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L10	L9 with l3	772	L10
L9	l4 with l7	12745	L9
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L7	dna or nucleic or plasmid	211272	L7
L6	L5 with l1	94	L6
L5	L4 with l3 with l2	456	L5
L4	polymer or microparticle or microsphere or nanoparticle or nanocapsule or microcapsule	1560829	L4
L3	encapsulat$	168773	L3
L2	ethanol or isopropyl or alcohol or PVA	848635	L2
L1	aqueous	932597	L1

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L8: Entry 3 of 4 File: USPT Feb 13, 2001

DOCUMENT-IDENTIFIER: US 6187319 B1
TITLE: Cross-protective rotavirus vaccine

Detailed Description Text (63):
To encapsulate the DNA in PLG microparticles, the DNA was emulsified with PLG dissolved in dichloromethane, and this water-in-oil emulsion was emulsified with aqueous polyvinyl alcohol (an emulsion stabilizer) to form a (water-in-oil)-in-water double emulsion. This double emulsion was added to a large quantity of water to dissipate the dichloromethane, which resulted in the microdroplets hardening to form microparticles. These were harvested by centrifugation, washed several times to remove the polyvinyl alcohol and residual solvent, and finally lyophilized. The microparticles containing DNA had a mean diameter of 0.5 .mu.m. To test for DNA content, the microparticles were dissolved in 0.1 M NaOH at 100.degree. C. for 10 minutes. The A.sub.260 was measured and DNA calculated from a standard curve. Incorporation of DNA into microparticles was 1.76 g to 2.7 g DNA per mg PLG for the VP6 DNA vaccines and 1.75 g to 3.61 g per mg PLG for the plasmid control.

then frozen and lyophilized as in Example 2. The final concentration of the excipients in the microspheres upon resuspension at 50 mg/ml was 0.1% Tween 80, 5% D-sorbitol, 5% D-mannitol, or 0.5% carboxymethylcellulose (CMC). DNA was extracted from the microspheres and analyzed on an agarose gel.

L11: Entry 2 of 10 File: PGPB Dec 5, 2002

DOCUMENT-IDENTIFIER: US 20020182258 A1
TITLE: Microparticles for delivery of nucleic acid

Summary of Invention Paragraph (33):
[0032] The first or second solution can optionally include a surfactant, a DNA-condensing agent, or a stabilizer compound (e.g., 1-10% dextrose, trehalose, sucrose, dextran, or other carbohydrates, polyvinyl alcohol, cyclodextrin, hexadecyltrimethylammonium bromide, Pluronic F-68 (Sigma-Aldrich Co., St. Louis, Mo.), another lipid, or dextran sulfate) that can stabilize the nucleic acid or emulsion by keeping the nucleic acid supercoiled during encapsulation and throughout the microparticle formation.

Detail Description Paragraph (111):
[0173] The results indicate that ethanol precipitation of DNA prior to encapsulation in microspheres resulted in increased incorporation ranging from 31% to greater than 56%, representing a 44-62% increase in the amount of encapsulated DNA.

Detail Description Paragraph (116):
[0178] The data show that ethanol precipitation increased the amount of DNA encapsulated in microspheres by 29-59%. The effect was demonstrated to hold regardless of size and preparation technique.

Detail Description Paragraph (124):
[0184] Plasmid DNA was resuspended in TE buffer following ethanol-precipitation, in an attempt to increase DNA stability. The microspheres were then prepared as described in Example 2. DNA was extracted from the microspheres and analyzed by agarose gel electrophoresis. One lane was loaded with the input plasmid (pIiPLPLR); another lane with the plasmid DNA following ethanol precipitation, resuspension in water, and encapsulation in microspheres; and still another lane with the plasmid DNA following ethanol precipitation, resuspension in TE buffer, and encapsulation in microspheres. The results indicated that the amount of supercoiled DNA within microspheres was increased by resuspension in TE buffer.

Detail Description Paragraph (129):
[0189] An experiment was carried out to determine the effect of Ph on encapsulation (the Ph of the EDTA, Tris, and TE solutions in the previous experiment were all similar). Microspheres were made by encapsulating DNA that had been ethanol precipitated and resuspended in Tris of different Ph, or in phosphate buffered saline (PBS). The DNA was extracted after lyophilization of the particles, and analyzed on agarose gel. The results indicated that there was a significant Ph effect on the stability of encapsulated DNA. Resuspension of the DNA in water (Ph 6.5), PBS (Ph 7.3), and Tris (Ph 6.8) all led to a decrease in the ratio of supercoiled DNA relative to total DNA within the microspheres. Increasing the Ph to 7.5 or higher had a positive effect on the amount of supercoiling, suggesting that basic Ph levels are important for maintaining DNA stability. Increased Ph also had an effect on encapsulation efficiency:

Detail Description Paragraph (140):
[0198] To determine whether or not excipient compounds have an adverse effect on encapsulated plasmid DNA, microspheres were prepared from ethanol-precipitated DNA following the protocol in Example 2, with the exception that prior to lyophilization, the microspheres were resuspended in solutions containing excipients. Each sample was